JOURNAL OF BACTERIOLOGY, Sept. 1979, p. 1085-1088
0021-9193/79/09-1085/04$02.00/0

Vol. 139, No. 3

Interaction Between Mutant Alleles of *araC* of the *Escherichia coli* B/r L-Arabinose Operon

DAVID E. SHEPPARD,[1]* MARIANNE ELEUTERIO,[2] AND BARRY FALGOUT[1]†

School of Life and Health Sciences, University of Delaware, Newark, Delaware 19711,[1] and Department of Biology, West Chester State College, West Chester, Pennsylvania 19380[2]

Received for publication 29 June 1979

Strains were constructed that contain mutational alterations affecting two distinct functional domains within the *araC* gene protein. The $araC^{i}$ (catabolite repression insensitivity) and $araC^{h}$ (catabolite repression hypersensitivity) mutations were used to alter the catabolite repression sensitivity domain, and mutation to D-fucose resistance was used to alter the inducer binding domain. $araC^{h}$, D-fucose-resistant double mutants never exhibited constitutive *ara* operon expression, whereas all of the $araC^{i}$, D-fucose-resistant double mutants did exhibit constitutivity. When L-arabinose was used as an inducer, most of the double mutants exhibited the sensitivity to catabolite repression associated with the $araC^{i}$ or $araC^{h}$ mutation. However, when D-fucose was used as an inducer, changes in sensitivity to catabolite repression were observed that were attributed to interactions between the two protein domains. The roles of catabolite activator protein and *araC* gene protein in the induction of the *araBAD* operon were discussed.

The L-arabinose operon (5, 10) consists of three structural genes that code for the enzymes necessary for the initial steps in L-arabinose catabolism (*araA*, L-arabinose isomerase; *araB*, L-ribulokinase; and *araD*, L-ribulose-5-phosphate epimerase). A controlling region adjacent to gene *araB* consists of an operator region, *araO*, and an initiator region, *araI*. The regulatory gene, *araC*, is under separate control and codes for a gene product which, in the absence of inducer (L-arabinose), is believed to interact with the operator region to prevent *araBAD* expression. Upon addition of inducer, the repressor form of the *araC* gene product is believed to be removed from the operator and converted to an activator, which then can interact with the initiator region to facilitate transcription of the *araBAD* operon. Finally, efficient transcription of the *araBAD* operon requires CAP (catabolite activator protein) and cAMP (cyclic AMP) and thus is subject to both transient and permanent catabolite repression. Whereas in the *lac* system the CAP-cAMP complex appears to interact solely with a distinct region of the DNA of the promoter region (4, 9), some genetic evidence exists that the CAP-cAMP complex may interact with the regulatory protein of the *ara* operon (*araC* gene product) as well as with DNA sequences within the arabinose initiator region (1, 3, 7, 8, 10, 14). The evidence for the interaction of CAP-cAMP with the *araC* gene protein is based upon the properties of two classes of mutants in the *araC* gene, both of which exert their mutant phenotypes in *trans* via the *araC* protein. $araC^{h}$ mutants result in hypersensitivity to catabolite repression, a phenotype that can be partially reversed by addition of cAMP (6, 14). $araC^{i}$ mutants, on the other hand, can achieve significant levels of *ara* operon expression in the absence of CAP-cAMP and are insensitive to catabolite repression (7, 8). Mutations in gene *araC* clearly can alter sensitivity to catabolite repression. Although other interpretations are possible, these results could be explained if the CAP-cAMP complex directly interacted with the *araC* protein in the formation of the transcriptional initiation complex. According to this model, $araC^{h}$ protein might have a lowered affinity for CAP-cAMP whereas $araC^{i}$ protein would be altered such that CAP-cAMP is no longer required for the formation of the transcriptional initiation complex.

Functional domains within the *lac* repressor protein have recently been demonstrated using a combined genetic and protein chemistry approach (12). The region of the repressor protein that interacts with the *lac* operator is confined to the first 50 amino acids of the sequence. The remaining carboxy-terminal portion of the protein contains the structural information for (i)

† Present address: Department of Biology, Johns Hopkins University, Baltimore, MD 21218.

formation of the tetrameric repressor structure, (ii) the binding of inducer, and (iii) the configurational change that destroys the *lac* operator binding function after inducer binding.

The experiments to be described here were designed to obtain evidence for and describe the interactive properties of two functional domains within the *araC* protein: (i) the domain responsible for inducer binding, and (ii) the domain controlling sensitivity to catabolite repression. Mutational alterations were introduced into both domains as follows. First, strains were chosen that contained mutational alterations (either $araC^h$ or $araC^i$) in the catabolite repression sensitivity domain. These strains were then subjected to mutagenesis, and selection was carried out for D-fucose resistance. Previous studies have indicated that mutations to D-fucose resistance map in gene *araC* (the regulatory gene), can act *trans*, and can use D-fucose as a gratuitous inducer (2, 11). The latter observation suggests that the inducer binding domain has been altered in these mutants. Furthermore, many D-fucose-resistant mutants have been shown to be constitutive for *ara* operon expression. Thus the selection of D-fucose-resistant mutants from strains containing the $araC^h$ or $araC^i$ mutations should provide strains with alterations in two different domains of the *araC* gene protein.

A total of 20 independent D-fucose-resistant mutants were derived from two $araC^h$-containing strains (RG0603 and RG0828), and 20 independent D-fucose-resistant mutants were derived from an $araC^i$-containing strain (ME7573). Steady-state rates of *ara* operon expression were determined by measuring L-arabinose isomerase activity (13). The induced rate of *ara* operon expression for 37 of these strains ranged from 40 to 150 U of isomerase, indicating that the efficiency of activatory function among these mutants can vary widely. The pattern of constitutive operon expression by these mutants, however, was very different. None of the 18 D-fucose-resistant mutants derived from the two $araC^h$ mutants that were tested exhibited any constitutive activity at all. Each of the 19 D-fucose-resistant mutants derived from the $araC^i$ strain, however, exhibited some constitutive operon expression ranging from 5 to 65 U of isomerase. Mutation to D-fucose resistance normally allows a portion of the repressor form of the *araC* protein to be converted to activator in the absence of inducer (2, 11, 13). This of course results in constitutive expression of the *ara* operon. The actual level of constitutivity is uniquely defined by the properties of each particular D-fucose-resistant mutant (2). Clearly the presence of the $araC^h$ mutation somehow precludes the spontaneous conversion of the *araC* repressor to activator when a D-fucose-resistant mutation is introduced into the inducer binding domain. Our current understanding of the *ara* activator protein does not permit us to definitively explain this striking effect.

Each of 20 different D-fucose-resistant mutants derived from the $araC^i$ mutant strain was found to exhibit *ara* operon inducibility with D-fucose. Inducible levels ranged from 18 to 90 U of isomerase, with an average value of 54 U. D-Fucose inducibility among 20 different D-fucose-resistant mutants derived from the $araC^h$ mutant strain exhibited a quite different pattern. Fifteen of the twenty mutants examined exhibited less than 10 U of operon expression in the presence of D-fucose, with an average value for all mutants of 19 U. The ability of D-fucose to serve as a gratuitous inducer is clearly impaired when the activator protein also contains an $araC^h$ mutation.

Each of the D-fucose-resistant mutants derived from the $araC^i$ and $araC^h$ strains was examined for sensitivity to catabolite repression by glucose under growth conditions where L-arabinose was serving as inducer. The average catabolite repression for D-fucose-resistant mutants derived from $araC^h$ mutant strains was 92%, whereas the average value for D-fucose-resistant mutants derived from the $araC^i$ mutant strains was only 32%. This is in comparison to 67% catabolite repression for the wild-type strain. Thus, in the majority of mutants, alteration of the inducer binding domain by mutation to D-fucose resistance does not alter the phenotypic expression of the $araC^i1$ and $araC^h$ mutations in the catabolite repression sensitivity domain of the *araC* gene protein when L-arabinose is used as inducer.

There were five D-fucose-resistant mutants derived from the $araC^h$ mutant strains that were unusual in that they exhibited only 60 to 90% catabolite repression. These were the same strains that exhibited significant operon inducibility with D-fucose. Two of these strains and two D-fucose-resistant mutants derived from strain ME7573 ($araC^i1$) were used to determine whether the choice of inducer would change the sensitivity of these strains to catabolite repression by D-glucose. In one set of experiments, D-fucose occupied the inducer binding site, whereas in the second set of experiments, L-arabinose interacted with the inducer binding domain. The results (Table 1) indicate that two of the D-fucose-resistant mutants isolated from $araC^h$-containing strains exhibit intermediate levels of catabolite repression when L-arabinose is inducer, but exhibit almost complete catabolite repression when D-fucose is used as inducer. Two D-fucose-resistant mutants derived from

TABLE 1. *D-Fucose-resistant mutants derived from araC^h and araC^i mutant strains: differences in sensitivity to catabolite repression after induction with L-arabinose (the normal inducer) and D-fucose (a gratuitous inducer)*

Strain	Differential rate[a]		Catabolite repression (%)	Differential rate[a]		Catabolite repression (%)
	+ara	+ara, +glu		+fuc	+fuc, +ara	
UP1000 (*araC*$^+$)	61.0	20.0	67.2			
ME7573 (*araC*^{i}*1*)	48.8	48.8	<0.1			
RG0603 (*araC*^{h}*602*)	54.3	2.1	96.1			
RG0828 (*araC*^{h}*828*)	70.2	1.6	97.7			
DS2024 (*araC*^{h}*602* D-Fuc^r-4)	73.5	40.4	45.0	133.3	1.0	99
DS2004 (*araC*^{h}*828* D-Fuc^r-14)	66.6	22.5	66.2	141.6	5.0	96.5
DS2052 (*araC*^{i}*1* D-Fuc^r-12)	68.8	68.8	<0.1	64.5	5.8	91.0
DS2053 (*araC*^{i}*1* D-Fuc^r-13)	36.6	36.6	<0.1	42.9	72.9	−69.9

[a] The differential rate of isomerase synthesis was determined over approximately one cell generation in cultures that had been grown previously for two generations in minimal salts-casein hydrolysate medium containing 2.2×10^{-2} M L-arabinose or 2.2×10^{-2} M D-fucose. D-Glucose at 2.2×10^{-2} M was added as indicated.

the *araC*^{i}*1*-containing strain both exhibit complete insensitivity to catabolite repression when induced with L-arabinose. However, when D-fucose is used as an inducer, one mutant (DS2052) exhibits hypersensitivity to catabolite repression, whereas the other (DS2053) exhibits a 1.7-fold increase in operon expression when D-glucose is added to the growth medium. These results suggest that, when D-fucose and L-arabinose can both interact with the inducer binding domain as inducers, they can produce significantly different configurational effects that ultimately affect the domain controlling sensitivity to catabolite repression.

Many of the observations reported here could be explained if the CAP-cAMP complex interacted directly with the catabolite repression sensitivity domain of the *araC* protein and assisted it in forming the activator protein-initiator DNA complex. *araC*^{h} mutations could then be explained as alterations of a CAP-cAMP binding domain that result in a lower affinity for CAP-cAMP, and the *araC*^{i}*1* mutation could be an alteration that somehow negates the requirement for CAP-cAMP binding before the formation of the transcription initiation complex. The absence of constitutive operon expression in any of the D-fucose-resistant mutants derived from a strain containing an *araC*^{h} mutation could be explained if CAP-cAMP is required for conversion of repressor to activator. The reduced affinity of CAP-cAMP for the binding domain of the *araC*^{h} protein might block the otherwise spontaneous conversion of repressor to activator. The interactions between the inducer binding domain and the catabolite repression sensitivity domain could be explained if the binding of inducer, either L-arabinose or D-fucose, can alter the affinity of the *araC* protein for CAP-cAMP. This work would suggest that future models describing the function of the *araC* protein must consider the existence of at least three domains: an inducer binding domain, a catabolite repression sensitivity domain (possibly a CAP-cAMP binding site), and an operator-initiator DNA binding domain.

This investigation was supported by research grant PCM 75-03400-A02 from the National Science Foundation and by UNIDEL Foundation Grant: Human Genetics.

The technical assistance of Chris Savage is gratefully acknowledged.

LITERATURE CITED

1. **Bass, R., L. Heffernan, K. Sweadner, and E. Englesberg.** 1976. The site for catabolite deactivation in the L-arabinose BAD operon in *E. coli* B/r. Arch. Microbiol. **110**:135-143.
2. **Beverin, S., D. E. Sheppard, and S. S. Park.** 1971. D-Fucose as a gratuitous inducer of the L-arabinose operon in strains of *Escherichia coli* B/r mutant in gene *araC*. J. Bacteriol. **107**:79-86.
3. **Colome, J., G. Wilcox, and E. Englesberg.** 1977. Constitutive mutations in the controlling site region of the *araBAD* operon of *Escherichia coli* B/r that decrease sensitivity to catabolite repression. J. Bacteriol. **129**: 948-958.
4. **Dickson, R., J. Abelson, W. M. Barnes, and W. S. Reznikoff.** 1975. Genetic regulation: the *lac* control region. Science **187**:27-35.
5. **Englesberg, E., and G. Wilcox.** 1974. Regulation: positive control. Annu. Rev. Genet. **8**:219-242.
6. **Gendron, R. P., and D. E. Sheppard.** 1974. Mutations in the L-arabinose operon of *Escherichia coli* B/r that result in hypersensitivity to catabolite repression. J. Bacteriol. **117**:417-421.
7. **Heffernan, L., R. Bass, and E. Englesberg.** 1976. Mutations affecting catabolite repression of the L-arabinose regulon in *Escherichia coli* B/r. J. Bacteriol. **126**:1119-1131.
8. **Heffernan, L., and G. Wilcox.** 1976. Effect of *araC* gene product on catabolite repression in the L-arabinose regulon. J. Bacteriol. **126**:1132-1135.
9. **Hopkins, J.** 1974. A new class of promoter mutations in the *lac* operon of *E. coli*. J. Mol. Biol. **87**:715-724.
10. **Lee, N.** 1978. Molecular aspects of *ara* regulation, p. 389-409. *In* J. H. Miller and W. S. Reznikoff (ed.), The operon. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
11. **Nathanson, N., and R. Schleif.** 1975. Paucity of sites

mutable to constitutivity in the *araC* activator gene of the L-arabinose operon of *Escherichia coli*. J. Mol. Biol. **96**:185–199.

12. **Ogata, R., and W. Gilbert.** 1978. An amino terminal fragment of *lac* repressor binds specifically to *lac* operator. Proc. Natl. Acad. Sci. U.S.A. **75**:5851–5854.
13. **Rock-MacInnes, K., D. E. Sheppard, and B. Falgout.** 1978. Regulatory properties of *araC*$^{-}$ mutants in the L-arabinose operon of *Escherichia coli* B/r. J. Bacteriol. **133**:178–184.
14. **Sheppard, D. E., and M. Eleuterio.** 1976. Hypersensitivity to catabolite repression in the L-arabinose operon of *Escherichia coli* B/r is *trans* acting. J. Bacteriol. **126**:1014–1016.